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(54) Title: THERAPEUTIC NUCLEOSIDES

(57) Abstract

The present invention relates to the use of 2'-deoxy-4'-thioribonucleosides and physiologically acceptable salts, esters or salts of such esters thereof, for the manufacture of a medicament for the treatment or prophylaxis of retrovirus, cytomegalovirus, varicella zoster virus, Epstein-Barr virus, human herpes virus 6, hepatitis viral infections, including hepatitis B, coxsackie virus and hepatitis C virus infections.

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THERAPEUTIC NUCLEOSIDES

The present invention relates to the use of 2'-deoxy-4'-thioribonucleosides and physiologically acceptable salts, esters or salts of such esters thereof, for the manufacture of a medicament for the treatment or prophylaxis of retrovirus, cytomegalovirus, varicella zoster virus, Epstein-Barr virus, human herpes virus 6, hepatitis viral infections, including hepatitis B, coxsackie virus and hepatitis C virus infections.

Of the DNA viruses, those of the herpes group are the sources of the most common viral illnesses in man. Most of the herpes viruses are able to persist in the host's neural cells; once infected, individuals are at risk of recurrent clinical manifestations of infection which can be both physically and psychologically distressing.

Varicella zoster virus (VZV) is a herpes virus which causes chickenpox and shingles. Chickenpox is the primary disease produced in a host without immunity and in young children is usually a mild illness characterised by a vesicular rash and fever. Shingles or zoster is the recurrent form of the disease which occurs in adults who were previously infected with varicella-zoster virus. The clinical manifestions of shingles are characterised by neuralgia and a vescicular skin rash that is unilateral and dermatomal in distribution. Spread of inflammation may lead to paralysis or convulsions. Coma can occur if the meninges becomes affected. In immunodeficient patients VZV may disseminate causing serious or even fatal illness. VZV is of serious concern in patients receiving immunosuppressive drugs for transplant purposes or for treatment of malignant neoplasia and is a serious complication of patients with Acquired Immune Deficiency Syndrome (AIDS) due to their impaired immune system.

In common with other herpes viruses, infection with CMV leads to a lifelong association of virus and host and, following a primary infection, virus may be shed for a number of years. Congenital infection following infection of the mother during pregnancy may give rise to clinical effects such as death or gross disease (microcephaly, hepatosplenomegaly, jaundice, mental retardation), retinitis leading to blindness or, in less severe forms, failure to thrive, and susceptibility to chest and ear infections. CMV infection in patients who are immunocompromised for example as a result of malignancy, treatment with immunosuppressive drugs following transplantation or infection with Human Immunodeficiency Virus (HIV) may give rise to retinitis,

pneumonitis, gastrointestinal disorders and neurological diseases. CMV infection in AIDS patients is a predominant cause of morbidity as it is present in a latent form in 50-80% of the adult population and can be re-activated in immunocompromised patients.

Epstein-Barr virus (EBV) causes infectious mononucleosis and hairy leukoplakis, and is also suggested as the causative agent of human cancer, such as nasopharyngeal cancer, immunoblastic lymphoma, Burkitt's lymphoma.

Human herpes virus 6 (HHV6) has been shown to be a causative agent of kidney rejection and interstitial pneumonia in kidney and bone marrow transplant patients respectively. There is also evidence of repression of stem cell counts in bone marrow transplant patients.

Another group of viral pathogens of world-wide major importance are the hepatitis viruses, in particular hepatitis B virus (HBV). HBV is aetiologically associated with primary hepatocellular carcinoma and is thought to cause 80% of the world's liver cancer. In the United States more than ten thousand people are hospitalised for HBV illness each year, and average of 250 die with fulminant disease. The United States currently contains an estimated pool of 500,000-one million infectious carriers. Chronic active hepatitis generally develops in over 25% of carriers, and often progresses to cirrhosis. Clinical effects of infection with HBV range from headache, fever, malaise, nausea, vomiting, anorexia and abdominal pains. Replication of the virus is usually controlled by the immune response, with a course of recovery lasting weeks or months in humans, but infection may be more severe leading to persistent chronic liver disease outlined above.

Of the RNA viruses, retroviruses have assumed a particular importance in recent years. Retroviruses form a sub-group of RNA viruses which, in order to replicate, must first 'reverse transcribe' the RNA of their genome into DNA ('transcription' conventionally describes the synthesis of RNA from DNA). Once in the form of DNA, the viral genome may be incorporated into the host cell genome, allowing it to take advantage of the host cell's transcription/translation machinery for the purposes of replication. Once incorporated, the viral DNA is virtually indistinguishable from the host's DNA and, in this state, the virus may persist for the life of the cell.

A species of retrovirus, Human Immunodeficiency Virus (HIV), has been reproducibly isolated from humans with AIDS or with the symptoms that frequently precede AIDS. AIDS is an immunosuppressive or immunodestructive disease that predisposes subjects to fatal opportunistic infections. Characteristically, AIDS is associated with a progressive depletion of T-cells, especially the helper-inducer subset bearing the OKT^4 surface marker. HIV is cytopathic and appears to preferentially infect and destroy T-cells bearing the OKT^4 marker and it is now generally recognised that HIV is the etiological agent of AIDS.

Another RNA virus which has been recognised as the causative agent of an increasingly serious international health problem is the non-A, non-B hepatitis virus. At least 80% of cases of chronic post-transfusional non-A, non-B hepatitis have been shown to be due to the virus now identified as hepatitis C and this virus probably accounts for cirtually all cases of post-transfusional hepatitis in clinical settings where blood products are screened for hepatitis B. Whereas approximately half of the cases of acute hepatitis C infection resolve spontaneously over a period of months, the remainder become chronic and in many if not all such cases chronic active hepatitis ensues with the potential for cirrhosis and hepatocellular carcinoma. The structure of the hepatitis C virus genome has recently been alucidated and the virus has been characterised as a single stranded RNA virus with similarities to flaviviruses.

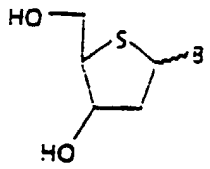
Coxsackie viruses belong to the enterovirus genus. They have a single stranded RNA genome contained in an icosahedral nucleocapsid. Coxsackie virus infection is increasingly recognised as a cause of primary myocardial disease in adults and children. Coxsackie infection is also associated with meningitis, pleurodynia, herpangia, hand-feet and mouth disease, respiratory disease, eye disease, diabetes and post-viral fatigue syndrome. In the latter case viral RNA has been detected in the muscle and in menocytes.

European Patent Specification Nos. EP 0 409 575 and EP 0 421 777 disclose certain 4'-thio-pyrimidine nucleosides and their use as antiviral agents.

It has now been found that certain 2'-deoxy-4'-thio-ribonucleosides, previously disclosed in International Patent Publication No. WO91/04033 for their use against herpes simplex virus types 1 or 2, are useful for the treatment or prophylaxis of

retrovirus, CMV, VZV, EBV, HHV6, hepatitis viral infections, particularly HBV, coxsackie virus and hepatitis C virus infections.

According to one aspect, the present invention provides the use of a compound of formula (I)



(I)

wherein ~~B indicates that B can be in either the α- or β-configuration, and B is a nitrogenous heterocyclic base selected from the group consisting of purine, 3-deazapurine, 7-deazapurine, 8-azapurine, and 2-azapurine bases; or a physiologically acceptable salt, ester or salt of an ester thereof, for the manufacture of a medicament for the treatment or prophylaxis of hepatitis viral infections, such as HBV.

By the term "purine base" is meant any purine derivative including, but not limited to, adenine (6-aminopurine), guanine (2-amino-6-oxopurine), 2,6-diaminopurine, 1-6-dihydro-6-oxopurine, and derivatives having a halogen attached to the C^2 heterocyclic carbon. By the term "3-deazapurine base" is meant any 3-deazapurine derivative including, but not limited to, 6-amino-3-deazapurine, 3-deaza-6-oxo-purine, and derivatives having an amino group or halogen attached to the C^2 heterocyclic carbon. By the term "7-deazapurine base" is meant any 7-deazapurine derivative including, but not limited to, 6-amino-7-deazapurine, 7-deaza-6-oxopurine, and derivatives having an amino group or a halogen attached to the C^2 heterocyclic carbon. By the term "8-azapurine base" is meant any 8-azapurine derivative including, but not limited to, 6-amino-8-azapurine, 8-aza-6-oxopurine, and derivatives having a halogen attached to the C^2 heterocyclic carbon. By the term "2-azapurine base" is meant any 2-azapurine derivative including, but not limited to, 6-amino-2-azapurine and 2-aza-6-oxopurine.

In a further aspect, the present invention provides the use of a compound of formula (I) above or a physiologically acceptable salt, ester or salt of an ester thereof in the manufacture of a medicament for the treatment or prophylaxis of CMV, VZV, EBV or

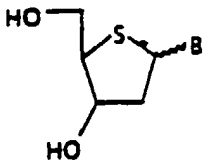
HHV6.

According to another aspect, the present invention provides the use of a compound of formula (I) or a salt, ester or salt of an ester thereof for the manufacture of a medicament for the treatment or prophylaxis of a retroviral infection, in particular an HIV infection.

Examples of retroviral infections which may be treated or prevented in accordance with the invention include human retroviral infections such as Human Immunodeficiency Virus (HIV), for example, HIV-1 or HIV-2 and Human T-cell Lymphotropic Virus (HTLV), for example, HTLV-1 or HTLV-II, infections. The compounds according to the invention are especially useful for the treatment of AIDS and related clinical conditions such as AIDS-related complex (ARC), progressive generalized lymphadenopathy (PGL), Kaposi's sarcoma, thrombocytopenic purpura, AIDS-related neurological conditions, such as multiple sclerosis or tropical paraperesis, and also anti-HIV antibody-positive and HIV-positive conditions, including such conditions in asymptomatic patients.

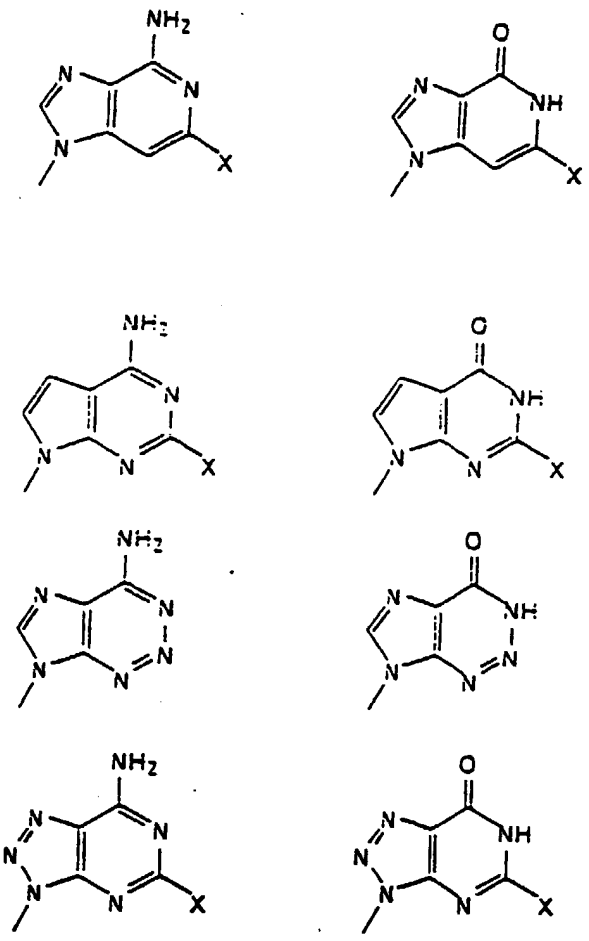
In yet a further aspect, present invention provides the use of a compound of formula (I) or salt, ester or a salt of an ester thereof in the manufacture of a medicament for the treatment or prophylaxis of a coxsackie virus or hepatitis C virus infection.

The compounds for use according to the invention include compounds of formula (I)



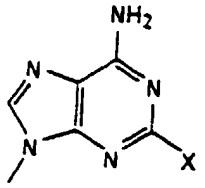
(I)

wherein ~B indicates that B can be in either the α- or β-configuration, and B is a member selected from the group consisting of the following nitrogenous heterocyclic bases:

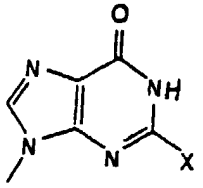


where X is H,NH_2 or halogen (Cl,Br,I,F).

According to a preferred aspect of the invention, the nitrogenous heterocyclic base is selected from the group consisting of the following purine bases:



and



where X is as hereinbefore defined.

Especially preferred compounds of formula (I), for use according to the invention, are 2'-deoxy-4'-thioadenosine and 2'-deoxy-4'-thioguanosine which are of particular use against HBV and CMV infections of animals, which term is intended to include humans, woodchucks and ducks.

The present invention also provides:-

a) A method for the treatment or prevention of the symptoms or effects of a hepatitis viral infection in an infected animal, for example, a mammal including a human, which comprises treating said animal with a therapeutically effective non-toxic amount of a compound of formula (I) or a salt, ester or salt of such ester thereof. According to a particular embodiment of this aspect of the invention, the hepatitis viral infection is an HBV infection.

b) A method for the treatment or prevention of the symptoms or effects of a CMV, VZV, EBV or HHV6 infection in an infected animal, for example, a mammal including a human, which comprises treating said host with a therapeutically effective non-toxic amount of a compound of formula (I) or a salt, ester or salt of such ester thereof.

c) A method for the treatment or prevention of the symptoms or effects of a retroviral infection for example, HIV in an infected animal, for example, a mammal including a human, which comprises treating said animal with a

therapeutically effective non-toxic amount of a compound of formula (I) or a salt, ester or salt of such ester thereof.

d) A method for the treatment or prevention of the symptoms or effects of a coxsackie virus or hepatitis C virus infection in an infected animal, for example, a mammal including a human, which comprises treating said animal with a therapeutically effective non-toxic amount of a compound of formula (I) or a salt, ester or salt of such ester thereof.

e) A method for the prophylaxis of an HBV, HIV, CMV, VZV, EBV, HHV6 coxsackie virus or hepatitis C virus infection in a animal, for example, a mammal including a human which comprises treating said animal with a therapeutically effective non-toxic amount of a compound according to the invention.

A further feature of the present invention is an anti-CMV,-VZV,-EBV,-HHV6, -hepatitis virus (eg. HBV), -retroviral (eg. HIV),-coxsackie virus or -hepatitis C virus pharmaceutical formulation comprising a compound of formula (I) or a physiologically acceptable salt, ester or salt of an ester thereof, together with at least one pharmaceutically acceptable carrier therefor.

Examples of the clinical conditions which may be treated in accordance with the invention include those caused by infections of heptatis viruses, particularly HBV, retroviruses such as HIV, coxsackie viruses, hepatitis C virus, CMV, VZV, EBV or HHV6 as hereinbefore described.

Preferred esters in accordance with the invention include carboxylic acid esters in which the non-carbonyl moiety of the carboxylic acid portion of the ester grouping is selected from straight or branched chain alkyl (for example, methyl, n-propyl, t-butyl, or n-butyl), cycloalkyl, alkoxyalkyl (for example, methoxymethyl), aralkyl (for example, benzyl), aryloxyalkyl (for example, phenoxymethyl), aryl (for example, phenyl optionally substituted by, for example, halogen, C_{1-4} alkyl, or C_{1-4} alkoxy), or amino; sulphonate esters, such as alkyl- or aralkylsulphonyl (for example, methanesulphonyl); amino acid esters (for example, L-valyl or L-isoleucyl); and mono-, di-, or tri-phosphate esters. In such esters, unless otherwise specified, any alkyl moiety present advantageously contains from 1 to 18 carbon atoms, particularly from 1 to 6 carbon

atoms, more particularly from 1 to 4 carbon atoms. Any cycloalkyl moiety present in such esters advantageously contains from 3 to 6 carbon atoms. Any aryl moiety present in such esters advantageously comprises a phenyl group. Any reference to any of the above compounds also includes a reference to a physiologically acceptable salt thereof.

Examples of physiologically acceptable salts of the compounds of formula (I) include salts derived from an appropriate base, such as an alkali metal (for example, sodium or potassium), an alkaline earth (for example, magnesium or calcium), ammonium and NX_4^+ (wherein X is C_{1-4} alkyl). Physiologically acceptable salts of an hydrogen atom or an amino group include salts of organic carboxylic acids such as acetic, lactic, fumaric, tartaric, malic, isethionic, lactobionic and succinic acids; organic sulphonic acids, such as methanesulphonic, ethanesulphonic, benzenesulphonic and p-toluenesulphonic acids, and inorganic acids, such as hydrochloric, sulphuric, phosphoric and sulphamic acids. Physiologically acceptable salts of a compound of an hydroxy group include the anion of said compound in combination with a suitable cation such as Na^+, NH_4^+ and NX_4^+ (wherein X is a C_{1-4} alkyl group).

The compounds according to the invention may be employed alone or in combination with other therapeutic agents for the treatment of the above infections or conditions. Combination therapies according to the present invention comprise the administration of at least one compound of the formula (I) or a physiologically acceptable salt, ester or salt of an ester thereof and at least one other physiologically acceptable ingredient. The active ingredient(s) and physiologically acceptable agents may be administered together or separately and, when administered separately this may occur simultaneously or sequentially in any order. The amounts of the active ingredient(s) and physiologically acceptable agent(s) and the relative timings of administration will be selected in order to achieve the desired combined therapeutic effect. Preferably the combination therapy involves the administration of one compound of the formula (I) or a physiologically acceptable salt, ester or salt of an ester thereof and one of the agents mentioned herein below.

Examples of such further therapeutic agents include agents that are effective for the treatment of HIV infections or associated conditions such as 3'-azido-3'-deoxythymidine (zidovudine), other 2',3'-dideoxynucleosides such as 2',3'-

dideoxycytidine, 2',3'-dideoxyadenosine and 2',3'-dideoxyinosine, carbovir, acyclic nucleosides (for example, acyclovir), 2',3'-didehydrothymidine, protease inhibitors such as N-tert-butyl-dehydro-2-[-2(R)-hydroxy-4-phenyl-3(S)-[[N-(2-quinolyl-carbonyl)-L-asparginyl]butyl]-(4aS,8aS)-isoquinoline-3(S)-carboxamide (Ro 31-8959), oxathiolane nucleoside analogues such as (-)-cis-1-(2-hydroxymethyl)-1,3-oxathiolan-5-yl)-cytosine (3TC) or cis-1-(2-(hydroxymethyl)-1,3-oxathiolan-5-yl)-5-fluoro-cytosine (FTC), 3'-deoxy-3'-fluorothymidine, 2',3'-dideoxy-5-ethynyl-3'-fluorouridine, 5-chloro-2',3'-dideoxy-3'-fluorouridine, (-)-cis-4-[2-amino-6-(cyclopropylamino)-9H-purin-9-yl]-2-cyclopentene-1-methanol, ribavirin, 9-[4-hydroxy-2-(hydroxymethyl)-but-1-yl]guanine (H2G), tat inhibitors such as 7-chloro-5-(2-pyrryl)-3H-1,4-benzodiazepin-2(H)-one (Ro5-3335), or 7-chloro-1,3-dihydro-5-(1H-pyrrol-2-yl)-3H-1,4-benzodiazepin-2-amine (Ro24-7429), interferons such as α-interferon, renal excretion inhibitors such as probenecid, nucleoside transport inhibitors such as dipyridamole; pentoxifylline, N-Acetylcysteine (NAC), Procysteine, α-trichosanthin, phosphonoformic acid, as well as immunodulators such as interleukin II, granulocyte macrophage colony stimulating factors, erythropoetin, soluble CD_4 and genetically engineered derivatives thereof. Examples of such further therapeutic agents which are effective for the treatment of HBV infections include carbovir, oxathiolane nucleoside analogues such as (-)-cis-1-(2-hydroxy-methyl)-1,3-oxathiolan-5-yl)-cytosine (3TC)) or cis-1-(2-(hydroxy-methyl)-1,3-oxathiolan-5-yl-5-fluoro-cytosine (FTC), 2',3'-dideoxy-5-ethynyl-3'-fluorouridine, 5-chloro-2',3'-dideoxy-3'-fluorouridine, 1-(β-D-arabino-furanosyl)-5-propynyluracil, acyclovir and interferons, such as α-interferon. Examples of further therapeutic agents which are effective for the treatment of herpes virus infections are acyclovir, 9-[4-hydroxy-2-(hydroxymethyl)butyl]-guanine (H2G), 9-[4-hydroxy-3-(hydroxymethyl)-but-1-yl)guanine (penciclovir), famciclovir, the diacetate ester of penciclovir, BVaraU, 1-(β-D-arabinofuranosyl)-5-propynyluracil, 2-[(2-amino-1,6-dihydro-6- oxo-9H-purin-9-yl)methoxy]-ethyl L-valinate, phosphonoformic acid and phosphonoacetic acid, ganciclovir, (S)-1-(3-hydroxy-2-phosphonylmethoxypropyl)-cytosine (HPMPC), Oxetanocin G, 2'-deoxy-5-iodo-uridine, E-5-2-bromovinyl-2'-deoxy-uridine (BVDU) and 9-(3-hydroxypropoxy) guanine.

More preferably the combination therapy involves the administration of one of the above-mentioned agents and a compound within one of the preferred sub-groups within formula (I) as described above. Most preferably the combination therapy involves the joint use of one of the above named agents together with one of the compounds of

formula (I) specifically named herein.

A compound of the invention (a compound of formula (I) or a physiologically acceptable salt, ester or salt of an ester thereof), also referred to herein as active ingredient, may be administered to a mammal including a human ("the recipient") by any route appropriate to the clinical condition to be treated; suitable routes include oral (including buccal and sublingual), rectal, nasal, topical (including buccal, sublingual and transdermal), vaginal and parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intrathecal and epidural). It will be appreciated that the preferred route may vary, for example, according to the age, weight and sex of the recipient and the nature and severity of the condition to be treated.

The amount of a compound for use according to the invention required for the treatment of each of the above indicated utilities and indications will depend on a number of factors including the severity of the condition to be treated and the identity of the recipient and will ultimately be at the discretion of the attendant physician.

In general, however, for each of these utilities and indications, a suitable, effective dose will be in the range 0.05 to 100 mg per kilogram body weight of the recipient per day, preferably in the range 0.1 to 50 mg per kilogram body weight per day and most preferably in the range 0.5 to 20 mg per kilogram body weight per day. An optimum dose is about 2 to 5 mg per kilogram body weight per day. For the treatment of HBV infections a suitable effective dose is preferably in the range of 0.05 to 20mg per kilogram body weight per day and for CMV infections the dose is preferably in the range of 0.5 to 20mg per kilogram per day. Unless otherwise indicated all weights of active ingredients are calculated as the parent compounds of the compounds according to the invention. In the case of an ester, salt, or salt of such ester of a compound according to the invention or a solvate of any thereof the figures would be increased proportionately. The desired dose is preferably presented as two, three, four, five, six, or more sub-doses administered at appropriate intervals throughout the day. These sub-doses may be administered in unit dosage forms, for example, containing from 1 to 1500 mg, preferably from 5 to 1000 mg, most preferably from 10 to 700 mg of active ingredient per unit dosage form. Alternatively, if the condition of the recipient so requires, the dose may be administered as a continuous infusion.

While it is possible for the active ingredient to be administered alone, it is preferable to present it as a pharmaceutical formulation. The formulations comprise at least one active ingredient, as defined above, together with one or more pharmaceutically acceptable carriers thereof and, optionally, one or more other therapeutic agents. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient.

Formulations of the invention include those suitable for administration by any of the aforementioned routes which may conveniently be presented in unit dosage form and may be prepared by any method well known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers, or both, and then, if necessary, shaping the product.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or suspension in an aqueous or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary, or paste or may be contained within liposomes.

A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder (for example, povidone, gelatin, hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (for example, sodium starch glycollate, cross-linked povidone, crossed-linked sodium carboxmethyl cellulose), or a surface-active or dispersing agent. Moulded tablets may be made by moulding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile or to be soluble or effervescent when

added to liquid. Tablets may optionally be provided with an enteric coating, to provide release in parts of the gut other than the stomach.

Formulations suitable for oral use may also include buffering agents designed to neutralise stomach acidity. Such buffers may be chosen from a variety of organic or inorganic agents such as weak acids or bases admixed with their conjugated salts.

A capsule may be made by filling a loose or compressed powder on an appropriate filling machine, optionally with one or more additives. Examples of suitable additives include binders such as povidone; gelatin, lubricants, inert diluents and disintegrants as for tablets. Capsules may also be formulated to contain pellets or discrete sub-units to provide slow or controlled release of the active ingredient. This can be achieved by extruding and spheronising a wet mixture of the drug plus an extrusion aid (for example microcrystalline cellulose) plus a diluent such as lactose. The spheroids thus produced can be coated with a semi-permeable membrane (for example ethyl cellulose, Eudragit WE30D) to produce sustained release properties.

An edible foam or whip formulation ideally comprises; 50-70% of an edible oil, particularly a vegetable oil, including corn oil, peanut oil, sunflower oil, olive oil and soybean oil; 2-10% of one or more surfactants particularly lecithin, polyols, polyol polymer esters including glyceryl fatty acid esters, polyglyceryl fatty acid esters (e.g. decaglycerol tetraoleate), or sorbitan fatty acid esters (e.g. sorbitan monostearate); 1-4% of a propellant which is suitable for ingestion, notably a compressed gas propellant especially nitrogen, nitrous oxide or carbon dioxide, or a gaseous hydrocarbon especially propane, butane or isobutane; 0.5-30% of one or more viscosity modifiers of particle size in the range 10-50 microns in diameter, particularly powdered sugars or colloidal silicon dioxide; and optionally 0.5-1% of one or more suitable, non-toxic colourings, flavourings or sweetners. The active ingredient is preferably present in such formulations in a concentration of 10-46%, advantageously 30%. An edible foam or whip formulation as described above may be prepared in a conventional manner, for example by mixing the edible oil, surfactant(s) and any other soluble ingredients, adding the viscosity modifier(s) and milling the mixture to form a uniform dispersion and suspension. The active ingredient is blended into the milled mixture until evenly dispersed. Finally, a metered quantity of propellant is incorporated to the mixture after said mixture has been measured into a suitable dispensing container.

Pharmaceutical formulations for topical administration according to the present invention may be formulated as an ointment, cream, suspension, lotion, powder, solution, paste, gel, spray, aerosol or oil. Alternatively, a formulation may comprise a dressing such as a bandage or adhesive plaster impregnated with active ingredients and optionally one or more excipients or diluents.

Compositions suitable for transdermal administration may be presented as discrete patches adapted to remain in intimate contact with the epidermis of the recipient for a prolonged period of time. Such patches suitably contain the active compound 1) in an optionally buffered, aqueous solution, 2) dissolved in an adhesive; or 3) dispersed in a polymer. A suitable concentration of the active compound is about 1% to 35%, preferably about 3% to 15%. As one particular possibility, the active compound may be delivered from the patch by iontophoresis as generally described in Pharmaceutical Research, 3(6), 318 (1986).

For infections of the eye or other external tissues, for example mouth and skin, the formulations are preferably applied as a topical ointment or cream containing the active ingredient in an amount of, for example, 0.075 to 20% w/w, preferably 0.2 to 15% w/w and most preferably 0.5 to 10% w/w. When formulated in an ointment, the active ingredients may be employed with either a paraffinic or a water-miscible ointment base. Alternatively, the active ingredients may be formulated in a cream with an oil-in-water cream base or as a water-in-oil base.

If desired, the aqueous phase of the cream base may include, for example, at least 40-45% w/w of a polyhydric alcohol, i.e. an alcohol having two or more hydroxyl groups such as propylene glycol, butane-1,3-diol, mannitol, sorbitol, glycerol and polyethylene glycol and mixtures thereof. The topical formulations may include a compound which enhances absorption or penetration of the active ingredient through the skin or other affected areas. Examples of such dermal penetration enhancers include dimethylsulphoxide and related analogues.

The oily phase of an emulsion formulation according to the invention may comprise merely an emulsifier (otherwise known as an emulgent), but desirably comprises a mixture of at least one emulsifier with a fat or an oil or with both a fat and an oil. Preferably, a hydrophilic emulsifier is included together with a lipophilic emulsifier

which acts as a stablilizer. It is also preferred to include both an oil and a fat. Together, the emulsifer(s) with or without stabilizer(s) make up the so-called emulsifying wax, and the wax together with the oil and/or fat make up the so-called emulsifying ointment base which forms the oily phase of the cream formulations.

Emulgents and emulsion stabilizers suitable for use in the formulation of the present invention include Tween 60, Span 80, cetostearyl alcohol, myristyl alcohol, glyceryl mono-stearate and sodium lauryl sulphate.

The choice of suitable oils or fats for the formulation is based on achieving the desired cosmetic properties, since the solubility of the active compound in most oils likely to be used in pharmaceutical emulsion formulations is very low. The cream should preferably be a non-greasy, non-staining and washable product with suitable consistency to avoid leakage from tubes or other containers. Straight or branched chain, mono- or dibasic alkyl esters such as di-isoadipate, isocetyl stearate, propylene glycol diester of cocomut fatty acids, isopropyl myristate, decyl oleate, isopropyl palmitate, butyl stearate, 2-ethylhexyl palmitate or a blend of branched chain esters known as Crodamol CAP may be used, the last three being preferred esters. These may be used alone or in combination depending on the properties required. Alternatively, high melting point lipids such as white soft paraffin and/or liquid paraffin or other mineral oils can be used.

Formulations suitable for topical administration to the eye also include eye drops wherein the active ingredient is dissolved or suspended in a suitable carrier, especially an aqueous solvent. The ingredient is preferably present in such formulations in a concentration of 0.5 to 20%, advantageously 0.5 to 10%, particularly about 1.5% w/w.

Formulations suitable for topical administration in the mouth include lozenges comprising the active ingredient in a flavoured material, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert material such as gelatin and glycerin, or sucrose and acacia; and mouth-washes comprising the active ingredient in a suitable liquid carrier.

Formulations for rectal administration may be presented as a suppository with a suitable base comprising for example cocoa butter or higher fatty alcohol (e.g. hard wax, European Pharmacopoeia) or triglycerides and saturated fatty acids (e.g.

Witepsol).

Formulations suitable for nasal administration wherein the carrier is a solid include a coarse powder having a particle size for example in the range 20 to 500 microns which is administered in the manner in which snuff is taken, i.e. by rapid inhalation through the nasal passage from a container of the powder held close up to the nose. Suitable formulations wherein the carrier is a liquid, for administration as a nasal spray or as nasal drops, include aqueous or oily solutions of the active ingredient.

Suitable formulations for administration by inhalation include fine particle dusts or mists which may be generated by means of various types of metered dose pressurised aerosols, nebulizers or insufflators.

For pulmonary administration via the mouth, the particle size of the powder or droplets is typically in the range 0.5 - 10μm, preferably 1 - 5μm, to ensure delivery into the bronchial tree. For nasal administration, a particle size in the range 10 - 500μm is preferred to ensure retention in the nasal cavity.

Metered dose inhalers are pressurised aerosol dispensers, typically containing a suspension or solution formulation of the active ingredient in liquified propellant. During use these devices discharge the formulation through a valve adapted to deliver a meteredvolume, typically from 10 - 150μm, to produce a fine particle spray containing the active ingredient. Suitable propellants include propane and butane, certain chlorofluorocarbon compounds, commonly referred to as "CFS's", for example, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, or mixtures thereof. The formulation may additionally contain co-solvents, for example ethanol, surfactants such as oleic acid or sorbitan trioleate, antioxidants and/or suitable flavouring agents.

Nebulizers are commercially available devices that transform solutions or suspensions of the active ingredient into an aerosol therapeutic mist either by means of accerleration of a compressed gas through a narrow venturi orifice, typically air or oxygen, or by means of ultrasonic agitation. Suitable formulations for use in nebulizers consist of the active ingredient in a liquid carrier and comprising up to 40% w/w of the formulation, preferably less than 20% w/w. The carrier is typically water or a dilute aqueous

alcholic solution, preferably made isotonic with body fluids by the addition of, for example, sodium chloride. Optional additives include preservatives if the formulation is not prepared sterile, for example methylhydroxybenzoate, antioxidants, flavouring agents, volatile oils, buffering agents and surfactants.

Suitable formulations for administration by insufflation include finely comminuted powders which may be delivered by means of an insufflator or taken into the nasal cavity in the manner of a snuff. In the insufflator, the powder is contained in capsules or cartridges, typically made of gelatin or plastic, which are either pierced or opened in-situ and the powder either presented to air drawn through the device upon inhalation or alternatively delivered by means of a manually operated pump. The powder employed in the insufflator consists either solely of the active ingredient or of a powder blend comprising the active ingredient, a suitable powder diluent such as lactose, and an optional surfactant. The active ingredient typically comprises from 0.1 - 100% w/w of the formulation.

Pressurised aerosol formulations for inhalation are preferably arranged so that each metered dose contains from 0.05 to 5 mg of a compound of the invention. Similarly, powder formulations for insufflations are so arranged that each unit dose contains from 0.5 to 50 mg. Solution or suspension formulations for nebulisation are arranged as to deliver doses between 1 and 1500 mg. The compounds according to the invention or formulations thereof may be administered by these devices once or several times daily, with one or several doses, for example three or four, being given on each occasion.

Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

Formulations suitable for parenteral administration include aqueous and non- aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for

example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Preferred unit dosage formulations are those containing a daily dose or sub-dose, as herein above recited, or an appropriate fraction thereof, of an active ingredient.

It should be understood that in addition to the ingredients particularly mentioned above, the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example those suitable for oral administration may include flavouring agents.

A compound of formula (I) may be prepared by any method known in the art for the preparation of a similar compound. Typically compounds of formula (I) may be prepared by the method described in International Patent Specification No. WO91/04033 the contents of which are incorporated herein by reference.

Alternatively, a compound of the invention wherein B is a purine base may be prepared enzymatically for example, with a transferase enzyme, such as, trans-N-deoxyribosylase. The trans-N-deoxyribosylase may be isolated by standard biochemical techniques from E. coli strain SS70-8/15 which expresses lactobacillus enzyme, available from the American Type Culture Collection (ATCC) Rockville, MD 20852-1776 from June 17, 1992 under Accession No. ATCC 69016.

The enzymatic synthesis of compounds of formula (I) may conveniently be carried out by the methods described in European Patent Specification 0 421 739.

Esters of the invention may be prepared in a conventional manner for example by treatment of the compounds of formula (I) with an appropriate esterifying agent, for example, by treatment with an appropriate acid halide, for example chloride or anhydride.

The compounds of formula (I), including esters thereof, may be converted into physiologically acceptable salts in a conventional manner, for example, by treatment with an appropriate base. An ester or salt of a compound of formula (I) may be

converted into the parent compound by, for example, hydrolysis.

For better understanding of the invention the following examples are given by way of illustration and should not be considered as limiting in anyway:-

Example A

Preparation of trans-N-deoxyribosylase (E.C. 2.4.2.6) from Escherichia coli

E. coli strain SS70-8/15 was grown overnight (15-20 hr) in a rich medium, such as Luria broth, containing 150 g/mL ampicillin. The bacteria were collected from the growth medium by centrifugation at 4°C and the cell pellet washed with cold, 100 mM sodium phosphate buffer, pH 6.0. A cell extract was prepared by resuspending the washed cell pellet with 0.6-0.8 volumes of cold, 100 mM sodium phosphate buffer followed by passage of the cell suspension through a French press at 12-14 Kpsi. Whole cells and cell debris were removed by centrifugation in a 70Ti rotor and 50 Krpm for 90 min. The supernatant obtained following centrifugation was the high speed supernatant (HSS). The A_{260} for the HSS was adjusted to equal 180 by addition of cold, 100 mM sodium phosphate buffer. The diluted HSS was adjusted to 0.2% PEI (polyethyleneimine), incubated at 4°C for 15-30 min and then centrifuged. The supernatant obtained following the PEI precipitation was adjusted to 30% saturation with respect to $(NH_4)_2SO_4$, incubated at 4°C for 60-90 min and then centrifuged to pellet the protein. The protein precipitated with 30% $(NH_4)_2SO_4$ was slowly dissolved in 100 mM sodium phosphate buffer (pH 6.0) and then dialyzed against 2 to 6 liters of the same buffer.

After dialysis, the precipitate that formed was removed by centrifugation. The supernatant containing enzyme was heated 5-10 min in a 60°C water bath followed by a 20 min incubation in a ice/water slurry. The precipitate that formed during the heat treatment step was removed by centrifugation. The supernatant contained trans-N-deoxyribosylase which was used for nucleoside synthesis.

The trans-N-deoxyribosylase activity of each enzyme preparation was quantitated using deoxyinosine and cytosine as substrates in the xanthine oxidase coupled assay system

described by Cardinaud, R. 1978. Nucleoside Deoxyribosyltransferase from Lactobacillus helveticus. Methods Enzymol. 51:446-455.

E. coli strain SS70-8/15 was deposited at the American Type Culture Collection, (ATCC) Rockville, MD 20852-1776 on June 17, 1992 under Accession No. ATCC 69016.

Example B

Enzymatic preparation of 2'-deoxy-4'-thio-purine nucleosides

An appropriately substituted purine base was added to 900 mL of pH 6.0 citrate buffer to give 1mM purine base solution. The buffer was prepared by addition of 9.46 g (45 mmol) of citric acid to 900 ml of distilled deionized water and adjusting the final pH to 6.0 with sodium hydroxide. An α/β mixture (1:1) of 2'-deoxy-4'-thiouridine (Secrist, J.A. III, et al. J. Med. Chem., 34, 2361-2366 (1991) incorporated herein by reference) was added to give a concentration of 5mM in β compound. Solution was achieved by heating the mixture to 50°C with sonication. Trans-N-deoxyribosylase (2051 units/mL) was added to a final concentration of 5 units of enzyme/mL of reaction. The reaction mixture was maintained at 50°C. Every day for four days an equivalent portion of purine base was added. After five days the enzyme was removed by ultrafiltration. The water was removed lyophilization. The resulting white powdery residue was slurried with methanol (50 mL) and filtered. The solid was rinsed thoroughly with methanol (3 x 100 mL, or until no substantial UV activity was present in the filtrate). The combined filtrates were slurried with Dowex Ag-1 (OH form) resin (200 mL) and filtered. The resin was rinsed with methanol until no UV activity was present in the filtrate. The solvent was removed with a rotary evaporator. The sticky residue was dissolved in 100 mL of methanol and silica gel (~20 mL) was added. The product was purified by flash chromatography, 5 x 30 cm column, 95:5 CH_2Cl_2:CH_3OH as eluant. The purified product was lyophilized from H_2O to give the nucleoside as a white powder.

Example C

2-Amino-9-(2-deoxy-4-thio-β-D-erythro-pentofuranosyl)-6-methoxy-9H-purine

2-Amino-6-methoxypurine, prepared from 2-amino-6-chloropurine (Aldrich Chemical Co., Milwaukee, WI 53233) and methanol, was added to pH 6.0 citrate buffer as described above in Example B.

^{1}N NMR (DMSO-d_6): δ 2.34-2.37 (m, 1), 2.52-2.58 (m, 1), 3.36-3.39 (m, 1), 3.54-3.58 (m, 1), 3.68-3.74 (m, 1), 3.96 (s, 3), 4.45-4.48 (m, 1), 5.13 (t, 1, J=5.5), 5.30 (d, 1, J=3.8), 6.13 (t, 1, J=6.9), 6.50 (s, 2), 8.20 (s, 1).
UV (pH=7) λmax 281 (ε=11.0); λmax 252 (ε=9.7); λmin 263 (ε=6.5); λmin 232 (ε10.3). (pH=13) λmax 281 (ε=10.4); λmax 252 (ε=9.0); λmin 263 (ε6.0); λmin 233 (ε=6.0). MS (E1) m/z 298 (M+H).

Example D

2'-Deoxy-4'-thioguanosine

To a solution of 9-(2-deoxy-4-thio-β,D-ribofuranosyl)-2-amino-6-methoxypurine (100mg, 0.34mmol) in distilled H_2O (5mL) was added 1 M pH = 7 phosphate buffer (250μL) and adenosine deaminase (Boehringer Mannheim, 100μL). The reaction mixture was allowed to stir at room temperature for 2 days when TLC (8:2 CH_2Cl_2:CH_3OH) showed complete reaction. Methanol (5mL) and silica gel (5mL) were added, and the solvent was removed by rotary evaporation. The powdery residue was applied to the top of a 2.5 x 20cm flash column packed with 9:1 CH_2Cl_2:CH_3OH. Gradient elution with 9:1-8:2 CH_2Cl_2:CH_3OH gave the product as a white powder (50mg, 50%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.30-2.40 (m, 1), 2.52-2.62 (m, 1), 3.30-3.40 (m, 1), 3.46-3.58 (m, 1), 3.60-3.66 (m, 1), 4.46 (t, 1), 5.04 (t, 1), 5.50 (d, 1, J = 4), 5.94 (dd, 1, J = 4,8), 6.50 (s, 2), 8.08 (s, 1). MS (E1) m/z 284 (M+H).

Example E

2'-Deoxy-4'-thioadenosine

6-Chloropurine (Sigma Chemical Company, 155mg, 1mmol) was heated under reflux in a mixture of hexamethyldisilane (15mL) and trimethylsilylchloride (0.2mL) for one hour. The mixture was cooled, evaporated, and the silylated base taken up in dry acetonitrile (15mL). 1-Acetyl-3,5-di-o-p-toluoyl-2-deoxy-4-thio-ribose (prepared according to the method described in EPA-0 409 575, 214mg, 0.5mmol) was added at $0^{o}C$, followed by trimethylsilyltriflate (106μL). After one hour at $0^{o}C$, the mixture was quenched with 10% sodium bicarbonate solution (25mL), dichloromethane (30mL) added, the organic layer separated and dried (Na_2SO_4). Following evaporation, the residue was chromatographed over flash SiO_2, eluting with toluene/ETOAc (4:1), (3:1), (2:1) and (1:1).

Evaporation of the first nucleoside fractions eluted gave 9-(2-deoxy- 4-thio-3,5-di-o-p-toluoyl-β-D-ribofuranosyl)-6-chloropurine and its α-anomer.

This mixture was dissolved in ethanol (10mL) previously saturated with ammonia gas and stirred at ambient temperature for 18 hours. The mixture was evaporated, re-dissolved in a mixture of tetrahydrofuran (2mL) and ethanol (3mL, previously saturated with ammonia gas), and heated at $100^{o}C$ for 18 hours in a sealed tube. The solvent was evaporated, and the residue chromatographed over flash silica, eluting with $CHCl_3$/MeOH (6:1), (4:1) and (3:2). The fractions were analyzed by HPLC, those containing pure β anomer evaporated to give the product.

^1H NMR (200MHz,d4-MeOH): δ 8.50 (1H, s, H2 or H8), 8.20 (1H, s, H2 or H8), 6.45 (1H, pseudo triplet, H1', J-6Hz), 4.6 (1H, m, H3'), 3.95 (2H, m), 3.6 (1H, m), 2.75 (1H, m, $H2'_{\alpha}$), 2.6 (1H, m, $H2'_{\beta}$).

PHARMACEUTICAL FORMULATIONS

In the following formulation Examples, the "Active Ingredient" may be a compound of formula (I) or a physiologically acceptable salt, ester or salt of an ester thereof.

Example 1

Tablet Formulations

The following formulations A, B and C are prepared by wet granulation of the ingredients with a solution of povidone, followed by addition of the magnesium stearate and compression.

Formulation A

		mg/tablet	mg/tablet
(a)	Active ingredient	250	250
(b)	Lactose B.P.	210	26
(c)	Povidone B.P.	15	9
(d)	Sodium Starch Glycollate	20	12
(e)	Magnesium Stearate	5	3
		500	300

Formulation B

		mg/tablet	mg/tablet
(a)	Active ingredient	250	250
(b)	Lactose	150	-
(c)	Avicel PH 101	60	26
(d)	Povidone B.P.	15	9
(e)	Sodium Starch Glycollate	20	12
(f)	Magnesium Stearate	5	3
		500	300

Formulation C

	mg/tablet
Active ingredient	100
Lactose	200
Starch	50
Povidone	5

Magnesium stearate	4
	359

The following formulations, D and E, are prepared by direct compression of the admixed ingredients.

Formulation D

	mg/capsule
Active Ingredient	250
Pregelatinised Starch NF15	150
	400

Formulation E

	mg/capsule
Active Ingredient	250
Lactose	150
Avicel	100
	500

Formulation F (Controlled Release Formulation)

The formulation is prepared by wet granulation of the following ingredients with a solution of povidone followed by addition of the magnesium stearate and compression.

		mg/tablet
(a)	Active Ingredient	500
(b)	Hydroxypropylmethylcellulose (Methocel K4M Premium)	112
(c)	Lactose B.P.	53
(d)	Povidone B.P.C.	28
(e)	Magnesium Stearate	7
		700

Drug release takes place over a period of about 6-8 hours and is complete after 12 hours.

Example 2

Capsule Formulations

Formulation A

A capsule formulation is prepared by admixing the ingredients of Formulation D in Example 3 above and filling into two-part hard gelatin capsule.

Formulation B

		mg/capsule
(a)	Active ingredient	250
(b)	Lactose B.P.	143
(c)	Sodium Starch Glycollate	25
(d)	Magnesium Stearate	2
		420

Capsules are prepared by admixing the above ingredients and filling into two-part hard gelatin capsules.

Formulation C

		mg/capsule
(a)	Active ingredient	250
(b)	Macrogol 4000 BP	350
		600

Capsules are prepared by melting the Macrogol 4000 BP, dispersing the active ingredient in the melt and filling the melt into two-part hard gelatin capsules.

Formulation D

	mg/capsule
Active ingredient	250
Lecithin	100
Arachis Oil	100
	450

Capsules are prepared by dispersing the active ingredient in the lecithin and arachis oil and filling the dispersion into soft, elastic gelatin capsules.

Formulation E (Controlled Release Capsule)

The following controlled release capsule formulation is prepared by extruding ingredients (a), (b) and (c) using an extruder, followed by spheronisation of the extrudate and drying. The dried pellets are then coated with the release-controlling membrane (d) and filled into two-piece, hard gelatin capsules.

		mg/capsule
(a)	Active Ingredient	250
(b)	Microcrystalline Cellulose	125
(c)	Lactose BP	125
(d)	Ethyl Cellulose	13
		513

Example 3

Injectable Formulation

Formulation A

Active ingredient	0.200g
Hydrochloric acid solution, 0.1M	q.s. to pH 4.0 to 7.0
Sodium hydroxide solution, 0.1M	q.s. to pH 4.0 to 7.0

Sterile water	q.s. to 10ml

The active ingredient is dissolved in most of the water (35°-40°C) and the pH adjusted to between 4.0 and 7.0 using the hydrochloric acid or the sodium hydroxide as appropriate. The batch is then made up to volume with the water and filtered through a sterile micropore filter into a sterile amber glass vial 10ml and sealed with sterile closures and overseals.

Formulation B

Active ingredient	0.125 g
Sterile, pyrogen-free, pH 7 phosphate buffer, q.s. to 25 ml	

Example 4

Intramuscular injection

Active Ingredient		0.20 g
Benzyl Alcohol		0.10 g
Glycofurol 75		1.45 g
Water for Injection	q.s. to	3.00 ml

The active ingredient is dissolved in the glycofurol. The benzyl alcohol is then added and dissolved, and water added to 3 ml. The mixture is then filtered through a sterile micropore filter and sealed in sterile amber glass vials 3 ml.

Example 5

Syrup

Active ingredient	0.25 g
Sorbitol Solution	0.10 g
Glycerol	2.00 g
Sodium Benzoate	0.005 g
Flavour, Peach 17.42.3169	0.0125 ml

Purified Water	q.s. to	5.00 ml

The active ingredient is dissolved in a mixture of the glycerol and most of the purified water. An aqueous solution of the sodium benzoate is then added to the solution, followed by addition of the sorbitol solution and finally the flavour. The volume is made up with purified water and mixed well.

Example 6

Suppository

	mg/suppository
Active Ingredient	250
Hard Fat, BP (Witepsol H15 - Dynamit Nobel)	1770
	2020

One-fifth of the Witepsol H15 is melted in a steam-jacketed pan at 45°C maximum. The active ingredient is sifted through a 200 µm sieve and added to the molten base with mixing, using a Silverson fitted with a cutting head, until a smooth dispersion is achieved. Maintaining the mixture at 45°C, the remaining Witepsol H15 is added to the suspension and stirred to ensure a homogenous mix. The entire suspension is passed through a 250 µm stainless steel screen and, with continuous stirring, is allowed to cool to 40°C. At a temperature of 38°C to 40°C, 2.0g of the mixture is filled into suitable, 2 ml plastic moulds. The suppositories are allowed to cool to room temperature.

Example 7

Pessaries

	mg/pessary
Active ingredient	250
Anhydrate Dextrose	380
Potato Starch	363

Magnesium Stearate	7
	1000

The above ingredients are mixed directly and pessaries prepared by direct compression of the resulting mixture.

Antiviral Activity

(a) HBV Assay

Anti-HBV activity of compounds of formula (I) was determined with a high-capacity assay for assessing efficacy and cell growth inhibition (toxicity). Supernatants from growing HBV-producing cells (HepG2 2.2.15, P5A cell line) in 96-well plates are applied to microtiter plate wells which have been coated with a specific monoclonal antibody to HBV surface antigen (HBsAg). Virus particles present in the supernatants bind to the antibody and remain immobilized while other debris is removed by washing. These virus particles are then denatured to release HBV DNA strands which are subsequently amplified by the polymerase chain reaction and detected with a colorimetric hybrid-capture assay. Quantitation is achieved through fitting of a standard curve to dilutions of a cell supernatant with known HBV DNA content. By comparing HBV DNA levels of untreated control cell supernatants with supernatants containing a compound of formula (I), a measure of anti-HBV effectiveness is obtained.

The cells from which the supernatants were removed are fixed with ethanol and stained with a fluorescent DNA dye. The fluorescence signals of drug-treated cells are compared to those from untreated cells to assess the extent of growth inhibition toxicity.

Immunoaffinity Capture of HBV:

HBV producer cells, 2500 cells/well, were seeded in 96-well culture dishes in RPMI/10% fetal bovine serum/2mM glutamine (RPMI/10/2:). Media were replenished on days 1, 3, 5, and 7 with dilutions of a compound of formula (I) in RPMI/10/2 to a final volume of 150μL. Fifty μL of mouse monoclonal anti-

HBsAG antibody (10μg/mL) in PBS were added to each well of a round-bottom microtiter plate. After incubation overnight at 4°C, the solutions were aspirated and replaced with 100μL of 0.1% BSA in PBS. The plates were incubated for 2 hours at 37°C and the wells washed three times with PBS/0/01% Tween-20 (PBS/T) using a Nunc Washer. Ten μL of 0.035% Tween 20 in PBS were then added to all wells by Pro/Pette. Cell supernatants (25μL) containing extracellular virion DNA were transferred into wells by Pro/Pette; the final Tween concentration is 0.01%. Twenty-five μL HBV standard media dilutions in RPMI/10/2 were added to 2 rows of wells to serve as an internal standard curve for quantitation, and the plates were sealed and incubated at 4°C overnight. Samples were washed 5 times with PBS/T and 2 times with PBS, aspirating the last wash. Next, 25μL of 0.09N NaOH/0.01% NP40 were added to each well by Pro/Pette, and the sample wells were sealed and incubated at 37°C for 60 minutes. Samples were then neutralized with 25μL of 0.09N HC1/100 mM tris (pH 8.3).

Polymerase Chain Reaction (PCR):

Polymerase chain reaction (Saiki, R.K. et al., Science, 239 (4930) 487-91 (1988)) was carried out on 5μL samples, using a Perkin Elmer PCR kit. PCR is performed in "MicroAmp tubes" in final volume of 25μL. Primers were chosen from conserved regions in the HBV genome, as determined by alignment of several sequences. One primer is biotinylated at the 5-prime end to facilitate hybrid-capture detection of the PCR products. All primers were purchased from Synthecell Corp., Rockville, MD 20850.

Hybrid-Capture Detection of PCR Products:

PCR products were detected with horse radish peroxidase-labelled oligonucleotide probes (Synthecell Corp., Rockville, MD 20850), which hybridize to biotinylated strands of denatured PCR products directly in streptavidin-coated microtiter plate wells, using essentially the method of Holodiniy, M. et al., BioTechniques, 12 (1) 37-39 (1992). Modifications included the use of 25k PCR reaction volumes and sodium hydroxide denaturation instead of heat prior to hybridisation. Simultaneous binding of the

biotin moeity to the plate-bound streptavidin during the hybridization serves to "capture" the hybrids. Unbound labelled probes were washed away before colorimetric determination of the bound (hybridized) horse radish peroxidase. Quantities of HBV DNA present in the original samples ere calculated by comparison with standards. These values were then compared to those from untreated cell cultures to determine the extent of anti-HBV activity.

Growth Inhibition

After removal of the supernatants from the HBV-producing cells, the cells were fixed with 70% ETOH for 30 minutes, rinsed with serum-free RPMI 1640 and incubated with the DNA stain bisbenzimide (Calbiochem Corporation, La Jolla, CA) at 30μg/ml for 1 hour at 37°C in serum free - RPMI 1640.

Fluorescence values were measured and an evaluation of growth inhibition ($CCID_{50}$) was made by comparison to untreated control values.

Anti-HBV Efficacy

	HBV DNA Percent of Control	
Example	μM	Day 9
2'-deoxy-4'-thioguanosine	2	13.6
	0.4	10.6
	0.08	13.1
	0.016	14.8
	0.0032	25.5

IC_{50} (the median inhibitory concentration) is the amount of compound which produces a 50 percent decrease in HBV DNA. The IC_{50} of 4'-thio-2'-deoxyguanosine is less than 0.0032 μM (74.5% inhibition).

Growth Inhibition

2'-deoxy-4'-thioguanosine has an $CCID_{50}$ of 13μM.

$CCID_{50}$ is the amount of compound which produces a 50 percent decrease in cell growth compared to the growth of untreated cells.

(b) CMV Assay

Human cytomegalovirus (HCMV) was assayed in monolayers of MRC5 cells (human embryonic lung) in multiwell trays. The standard CMV strain AD 169 was used. Activity of compounds is determined in the plaque reduction assay, in which a cell monolayer is infected with a suspension of HCMV, and then overlaid with nutrient carboxymethyl cellulose in the form of a gel to ensure that there is no spread of virus throughout the culture. A range of concentrations of compound of known molarity was incorporated in the nutrient overlay. Plaque members at each concentration of a drug are expressed as percentage of the control and a dose-response curve is drawn.

	$IC_{50}\mu m$	$CCID_{50}\mu m$
2'-deoxy-4'-thioadenosine	>50<100	420.3

(c) VZV Assay

Clinical isolates of varicella zoster virus (VZV) were assayed in monolayers of MRC-5 cells. MRC-5 cells are derived from human embryonic lung tissue. A plaque reduction assay was used in which a suspension of the virus stock was used to infect monolayers of the cells in multiwell trays. A range of concentrations of the compound under test of known molarity was added to the wells. Plaque numbers at each concentration were expressed as percentages of

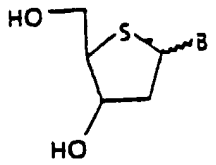
the control and a dose response curve was constructed. From these curves the 50% inhibitory concentration of each drug was determined.

(d) Cell Toxicity

Cell toxicity was assessed in cell growth inhibition assay. Subconfluent cultures of Vero cells grown on 96-well microtiter dishes were exposed to different dilutions of drug, and cell viability determined daily on replicate cultures using uptake of a tetrazolium dye (MTT). The concentration required for 50% inhibition of cell viability at 96 hours is termed $CCID_{50}$.

CLAIMS

1. Use of a compound of formula (I)



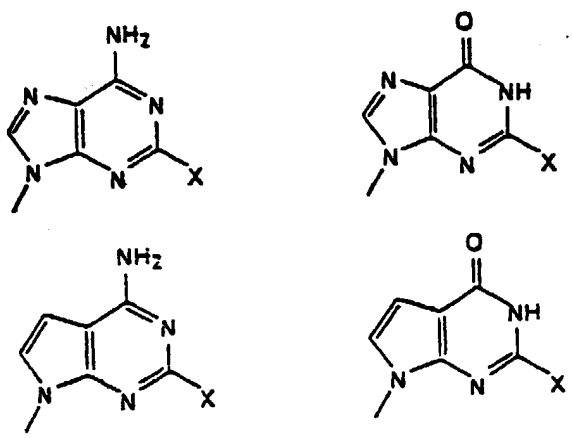
(I)

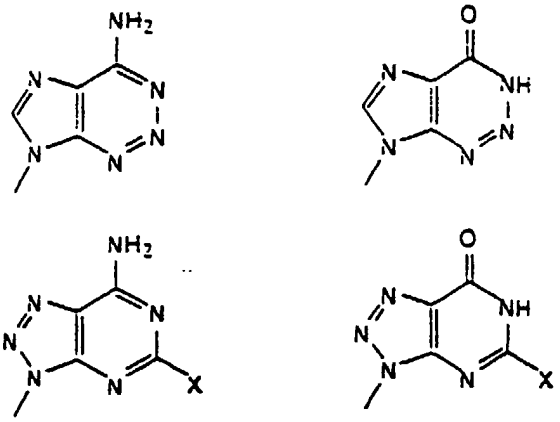
wherein ~B indicates that B can be in either the α- or β-configuration, and B is a nitrogenous heterocyclic base selected from the group consisting of purine, 3-deazapurine, 7-deazapurine, 8-azapurine, and 2-azapurine bases; or a physiologically acceptable salt, ester or salt of an ester thereof, for the manufacture of a medicament for the treatment or prophylaxis of an infection selected from a hepatitis virus, retrovirus, cytomegalovirus, varicella zoster virus, Epstein-Barr virus, human herpes virus 6, coxsackie virus and hepatitis C virus infections.

2. Use according to claim 1 wherein the hepatitis virus infection is a hepatitis B virus infection.

3. Use according to claim 1 wherein the infection is a cytomegalovirus infection.

4. Use according to any one of claims 1 to 3 wherein B is selected from

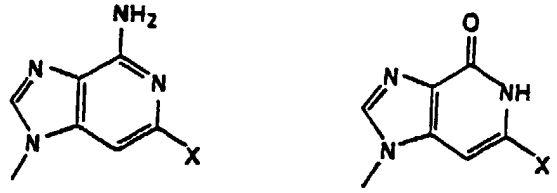




wherein ~~B indicates that B can be in either the α- or β-configuration and X is H,NH_2 or halogen;

and salts, esters and salts of esters thereof .

5. Use according to any one of claims 1 to 3 wherein B is selected from



wherein ~~B indicates that B can be in either the α- or β-configuration and X is H,NH_2 or halogen;

and salts, esters, and salts of esters thereof.

5. Use according to any one of claims 1 to 3 wherein the compound of formula (I) is 2'-deoxy-4'-thioadenosine; or a salt, ester or salt of such ester thereof.

6. Use according to any one of claims 1 to 3 wherein the compound of formula (I) is 2'-deoxy-4'-thioadenosine; or a salt, ester or salt of such ester thereof.

7. Use of a compound of formula (I) or a physiologically acceptable salt, ester or salt of an ester thereof as defined according to any one of claims 1 to 6 wherein the medicament is in a form suitable for oral administration.

8. Use of a compound of formula (I) or a physiologically acceptable salt, ester or salt of an ester thereof as defined according to any one of claims 1 to 6 wherein the medicament is in a form suitable for parental administration.

9. Use of a compound of formula (I) or a physiologically acceptable salt, ester or salt of an ester thereof as defined according to any one of claims 1 to 6 wherein the medicament is in a unit dosage form.

10. A method of treatment or prevention of the symptoms or effects of an infection selected from a hepatitis virus, retrovirus, cytomegalovirus, varicella zoster virus, Epstein-Barr virus, human herpes virus 6, coxsackie virus and hepatitis C virus infections in an infected animal which comprises administering to said animal a therapeutically effective amount of a compound of formula (I) or a salt, ester, or salt of an ester thereof as defined according to claim 1.

11. A method of treatment or prevention of the symptoms or effects of a hepatitis B virus infection in an infected animal which comprises administering to said animal a therapeutically effective amount of a compound of formula (I) or a salt, ester or salt of an ester thereof as defined according to claim 1.

12. A method of treatment or prevention of the symptons or effects of a cytomegalovirus infection in an infected animal which comprises administering to said animal a therapeutically effective amount of a compound of formula (I) or a salt, ester or salt of an ester thereof as defined according to claim 1.

13. An anti-hepatitis B virus pharmaceutical formulation comprising a compound of formula (I) or a salt, ester or salt of an ester thereof as defined according to claim 1, and a pharmaceutically acceptable carrier therefor.

14. An anti-cytomegalovirus pharmaceutical formulation comprising a compound of formula (I) or a salt, ester or salt of an ester thereof as defined according to claim 1, and a pharmaceutically acceptable carrier therefor.

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 93/01387

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 A61K31/70

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	A61K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
Y	WO,A,9 104 033 (SOUTHERN RESEARCH INSTITUTE) 4 April 1991 cited in the application see page 4, line 28 - page 7, line 11; example 17	1-12
X		13,14
Y	WO,A,9 116 333 (SOUTHERN RESEARCH INSTITUTE) 31 October 1991 see claims 1,2; example 28	1-12
Y	EP,A,0 409 575 (THE UNIVERSITY OF BIRMINGHAM) 23 January 1991 cited in the application see page 4, line 44 - page 5, line 50	1-12

° Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
13 OCTOBER 1993	25. 10. 93
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	TZSCHOPPE D. A.

Form PCT/ISA/210 (second sheet) (January 1985)

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

GB 9301387
SA 75992

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 13/10/93

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		WO-A-	9104982	18-04-91